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Decomposition of soybean grown under elevated concentrations of CO_2 and O_3

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Abstract

A critical global climate change issue is how increasing concentrations of atmospheric CO_2 and ground-level O_3 will affect agricultural productivity. This includes effects on decomposition of residues left in the field and availability of mineral nutrients to subsequent crops. To address questions about decomposition processes, a 2-year experiment was conducted to determine the chemistry and decomposition rate of aboveground residues of soybean (*Glycine max* (L.) Merr.) grown under reciprocal combinations of low and high concentrations of CO_2 and O_3 in open-top field chambers. The CO_2 treatments were ambient (370 μmol mol^{-1}) and elevated (714 μmol mol^{-1}) levels (daytime 12 h averages). Ozone treatments were charcoal-filtered air (21 nmol mol^{-1}) and nonfiltered air plus 1.5 times ambient O_3 (74 nmol mol^{-1}) 12 h day^{-1}. Elevated CO_2 increased aboveground postharvest residue production by 28–56% while elevated O_3 suppressed it by 15–46%. In combination, inhibitory effects of added O_3 on biomass production were largely negated by elevated CO_2. Plant residue chemistry was generally unaffected by elevated CO_2, except for an increase in leaf residue lignin concentration. Leaf residues from the elevated O_3 treatments had lower concentrations of nonstructural carbohydrates, but higher N, fiber, and lignin levels. Chemical composition of petiole, stem, and pod husk residues was only marginally affected by the elevated gas treatments. Treatment effects on plant biomass production, however, influenced the content of chemical constituents on an areal basis. Elevated CO_2 increased the mass per square meter of nonstructural carbohydrates, phenolics, N, cellulose, and lignin by 24–46%. Elevated O_3 decreased the mass per square meter of these constituents by 30–48%, while elevated CO_2 largely ameliorated the added O_3 effect. Carbon mineralization rates of component residues from the elevated gas treatments were not significantly different from the control. However, N immobilization increased in soils containing petiole and stem residues from the elevated CO_2, O_3, and combined gas treatments. Mass loss of decomposing leaf residue from the added O_3 and combined gas treatments was 48% less than the control treatment after 20 weeks, while differences in decomposition of petiole, stem, and husk residues among treatments were minor. Decreased decomposition of leaf residues was correlated with lower starch and higher lignin levels. However, leaf residues only comprised about 20% of the total residue biomass assayed so treatment effects on mass loss of total aboveground residues were relatively small. The primary influence of elevated atmospheric CO_2 and O_3 concentrations on decomposition processes is apt to arise from effects on residue mass input, which is increased by elevated CO_2 and suppressed by O_3.

Keywords: air pollution, chemical composition, decomposition, global change, *Glycine max*, nitrogen

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Introduction

Atmospheric CO_2 concentrations have increased from about 280 μmol mol^{-1} in 1800 to the present level of about 370 μmol mol^{-1}, and a doubling from preindustrial levels will occur in this century if current CO_2 emission rates continue (Prentice *et al*., 2001). Atmospheric CO_2 concentrations have a significant influence on agroecosystems because CO_2 is a primary substrate for photosynthesis, and elevated CO_2 stimulates growth of many C_3 crop species (Kimball, 1983; Rogers & Dahlman, 1993). Increasing atmospheric CO_2 concentration might also improve crop plant water balance and conserve soil hydrologic reserves (Allen *et al*., 1998; Polley, 2002).

Tropospheric O_3 is a natural component of the atmosphere originating primarily from photochemical reactions of nitrogen oxides and hydrocarbons with O_2. Ozone concentrations vary across regions, but often are significantly greater than background concentrations because of anthropogenic emissions of its precursors (Prather *et al*., 2001). Some of the most productive agricultural areas in the United States are exposed to elevated O_3. Ozone is highly phytotoxic. It inhibits photosynthesis and other physiological processes, causing significant growth and yield losses in soybean, cotton (*Gossypium hirsutum* L.), wheat (*Triticum aestivum* L.), and other crops, resulting in significant economic losses (Mauzerall & Wang, 2001; Adams & Horst, 2003). Tropospheric O_3 concentrations are projected to increase in future years as emissions of its precursors continue to grow (Prather *et al*., 2001).

Both CO_2 and O_3 affect plant chemistry. In soybean, for example, elevated CO_2 increased nonstructural carbohydrate levels whereas O_3 suppressed their accumulation (Reid *et al*., 1998; Ainsworth *et al*., 2002; Morgan *et al*., 2003). Leaf N concentrations were lower or not significantly different following exposure to elevated CO_2 or O_3 (Reid *et al*., 1998; Ainsworth *et al*., 2002; Morgan *et al*., 2003). Insoluble phenolic polymer concentrations increased in soybean leaves following chronic exposure to O_3 (Booker & Miller, 1998). It has been suggested that changes in plant productivity and chemistry caused by increasing atmospheric CO_2 and O_3 concentrations might affect crop residue decomposition and mineralization processes (Lambers, 1993; Islam *et al*., 2000; Torbert *et al*., 2000; Larson *et al*., 2002; Andersen, 2003; Loya *et al*., 2003). If so, there are long-term implications of such changes. Decomposition of biotic residues is the route by which much organic C is mineralized and returned to the atmosphere as CO_2. Over time, organic N, and other nutrients are mineralized and become available for other organisms. Residue input levels and composition also affect availability of mineral nutrients for other organisms and can lead to immobilization of mineral nutrients, such as N. Factors such as elevated atmospheric CO_2 and O_3 that affect plant productivity and chemistry might change rates of organic C and N turnover, and thus affect the global C cycle and ecosystem mineralization rates (Lambers, 1993; Islam *et al*., 2000; Torbert *et al*., 2000; Larson *et al*., 2002; Andersen, 2003; Loya *et al*., 2003).

Plant decomposition rates depend partly on the levels of polysaccharides, protein, organic acids, and phenolic compounds (particularly polyphenols and lignin) in plant residues (Haynes, 1986). However, it is unclear whether the changes in plant composition caused by elevated CO_2 concentrations will alter decomposition rates of crop residues (Norby *et al*., 2001). For example, laboratory microcosm experiments using soil amended with cotton, soybean, or grain sorghum (*Sorghum bicolor* (L.) Moench.) residue obtained from elevated CO_2 experiments suggested that increasing concentrations of atmospheric CO_2 will have little effect on C mineralization rates (Torbert *et al*., 1995; Henning *et al*., 1996; Booker *et al*., 2000). Percent recovery of soybean and sorghum residues was not affected by growth in elevated CO_2, although greater biomass production resulted in more residue and more C remaining after over-wintering (Prior *et al*., 2004). Enriched CO_2 levels did not affect the chemical composition of mature wheat stems or their biodegradation (Akin *et al*., 1995). Net N immobilization was greater in soils containing cotton, soybean, or sorghum residue from elevated CO_2 studies (Torbert *et al*., 1995, 1998), which suggested that nutrient cycling might be an important factor to consider (Torbert *et al*., 2000; Hu *et al*., 2001).

Ozone-induced changes in plant chemistry would be expected to slow decomposition rates although previous studies are limited to various forest and understory species. Mass loss from early-abscised cottonwood (*Populus deltoides* Marsh.) leaves and blackberry (*Rubus cuneifolus* Pursh.)/broomsedge (*Andropogon virginicus* L.) litter was lower for O_3-treated plants compared with control plants (Findlay & Jones, 1990; Kim *et al*., 1998). Decreased mass loss was attributed to higher concentrations of lignin and other phenolic polymers in O_3-treated plants. However, no O_3 effect on mass loss was found for yellow poplar (*Liriodendron tulipifera* L.), black cherry (*Prunus serotina* Ehrhart), sugar maple (*Acer saccharum* Marsh.), and eastern white pine (*Pinus strobus* L.) foliar residues (Boerner & Rebbeck, 1995; Scherzer *et al*., 1998). Exposure of Scots pine (*Pinus sylvestris* L.) to elevated CO_2, O_3, and their combination during three growing seasons did not affect subsequent decomposition rates of needles (Kainulainen *et al*., 2003).

In addition to biomass loading and residue chemistry, decomposition rates depend on belowground processes involving microbial, fungal, and faunal systems, all of which are influenced by soil type, moisture, and temperature. These belowground systems interact with roots and their exudates, which are affected by elevated CO_2 and O_3 (Larson *et al*., 2002; Andersen, 2003).

Clearly, questions remain about the effects of elevated CO_2 and O_3 on crop residue decomposition processes. Therefore, the objective of this study was to determine the chemistry, mineralization rates, and decomposition of aboveground residues of soybean plants exposed to reciprocal combinations of CO_2 and O_3 in a 2-year field experiment. It was hypothesized that decreases in N and increases in phenolic polymers with elevated CO_2 and O_3 would inhibit decomposition and mineralization. Lower nonstructural carbohydrate concentrations with O_3 were expected to contribute to slower decomposition whereas increases in nonstructural carbohydrate concentrations with elevated CO_2 could promote the process.

Materials and methods

Plant culture conditions, gas treatments, and sample preparation

The experiment was performed using postharvest, aboveground residues of soybean (cultivar Essex) grown during the 1999 and 2000 field seasons at a site 5 km south of Raleigh, NC (Booker *et al*., 2005). Seeds for the plants were treated with a commercial *Bradyrhizobium* preparation and planted on 24 May 1999 and 31 May 2000. The soil was an Appling sandy loam (clayey, kaolinitic, thermic, Typic Hapludult). The plants were sown in rows with 1 m spacing and with plant spacing of 5–8 cm. Plots were fertilized with K according to soil test recommendations. Plants were irrigated with soaker hoses as required to prevent visible signs of water stress. Plots were sprayed to control insects and spider mites with bifenthrin [(2-methyl-1,1-biphenyl-3-y1)-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate] (Whitmire Micro-Gen Research Laboratories Inc., St Louis, MO, USA) at 2.6 mL L^{-1} water and abamectin (avermectin B_1) (Syngenta Crop Protection Inc., Greensboro, NC, USA) at 0.32 mL L^{-1} water. (Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture or the North Carolina Agricultural Research Service.)

Table 1 Seasonal 12 h (08:00–20:00 hours EST) daily average CO_2 and O_3 concentrations in the 2-year experiment (1999 and 2000)

Growing season	Treatment	CO_2 (μmol mol^{-1})	O_3 (12 h average) (nmol mol^{-1})	O_3 (SUM06) (μmol mol^{-1} h^{-1})
1999	Control	370	24	0.88
	Elevated CO_2	700	21	0.57
	Elevated O_3	374	75	65.78
	Elevated CO_2 and O_3	700	75	66.14
2000	Control	367	20	0.18
	Elevated CO_2	730	20	0.06
	Elevated O_3	368	72	58.16
	Elevated CO_2 and O_3	726	72	59.84

Values for SUM06, the sum of all hourly average O_3 concentrations greater than or equal to 60 nmol mol^{-1} for the 3-month period between 15 June and 15 September, are also shown. Plants were treated with: (a) charcoal-filtered air and ambient CO_2 (control); (b) charcoal-filtered air plus approximately 344 μmol CO_2 mol^{-1} (elevated CO_2); (c) nonfiltered air plus 1.5 times ambient O_3 and ambient CO_2 (elevated O_3); and (d) nonfiltered air plus 1.5 times ambient O_3 and 344 μmol CO_2 mol^{-1} (elevated CO_2 and O_3). The O_3 and CO_2 treatments were administered 12 and 24 h day^{-1}, respectively, 7 days $week^{-1}$.

Plants were treated in cylindrical open-top field chambers, 3 m diameter × 2.4 m tall, from emergence to physiological maturity. Plants were exposed from mid-June to mid-October to reciprocal combinations of CO_2 and O_3. The treatment combinations were: (a) charcoal-filtered air and ambient CO_2 (control); (b) charcoal-filtered air plus 344 μmol CO_2 mol^{-1} (elevated CO_2); (c) nonfiltered air plus 1.5 times ambient O_3 and ambient CO_2 (elevated O_3); and (d) nonfiltered air plus 1.5 times ambient O_3 and 344 μmol CO_2 mol^{-1} (elevated CO_2 and O_3) (Table 1). Filtration of ambient air by activated charcoal lowered ambient O_3 concentrations to levels considered nonphytotoxic to soybean (Heagle, 1989). Air pollutants in nonfiltered air were primarily O_3 because concentrations of other major air pollutants such as NO_2 and SO_2 were below phytotoxic levels at our location.

Supplementary O_3 was generated by electrostatic discharge in dry O_2 (model GTC-1A, Ozonia North America, Elmwood Park, NJ, USA) and dispensed 12 h daily (08:00–20:00 hours EST) in a prescribed function based on historic measurements of ambient O_3 at our location. It was monitored at canopy height using UV photometric O_3 analyzers (model 49, Thermo

Environmental Instruments Co., Franklin, MA, USA). The O_3 analyzers were calibrated once every 2 weeks (model 49 PS calibrator, Thermo Environmental Instruments Co.). Carbon dioxide was dispensed from a 14 ton liquid receiver 24 h daily and was monitored at canopy height with infrared CO_2 analyzers (model 6252, Li-Cor Inc. Lincoln, NE, USA). The CO_2 monitors were calibrated once every 2 weeks with CO_2 standards.

Upon senescence of the plants, abscised leaves and petioles were collected from each chamber and air-dried in a greenhouse. At physiological maturity, the aboveground portion of all soybean plants were collected from each chamber and separated into remaining leaves, petioles, stems, and pod husks. The plant residues, which are defined as the plant matter returned to the field after grain harvest, were pooled by organ for each chamber, air-dried, and weighed.

Two randomly selected samples of each residue component (leaf, petiole, stem, and husk) obtained from each chamber were ground using a rotary mill. Samples ground to pass a 2.0 mm screen and retained by 0.5 mm screen were used in the laboratory microcosm assay. Samples that passed a 0.5 mm mesh screen were used in the plant chemistry assays. Randomly selected samples of unground leaf, petiole, stem, and husk residues were used in the litter bag assay. Petioles and stems were cut to 15 cm lengths before placement in the litter bags.

Plant chemistry assays

Starch and soluble sugars in each sample were determined enzymatically by the UV method (R-Biopharm Inc., Marshall, MI, USA). To solubilize starch, duplicate tissue samples (25 mg) were each mixed with 2.4 mL of dimethylsulfoxide and 600 μL of 8 N HCl in sealed polypropylene tubes for 60 min at 60 °C. Samples were then neutralized with 600 μL of 8 N NaOH and diluted to 15 mL with 112 mM citrate buffer (pH 4). Solutions were filtered, and 50 μL aliquots were assayed according to kit instructions. Results were expressed as D-glucose equivalents.

To determine total phenolic concentrations, duplicate tissue samples (50 mg) were extracted with 1 mL of 50% methanol (3 ×) for 5 min at room temperature with periodic mixing, centrifuged (16 000 × *g*), and the supernatants pooled by sample. Total phenolic concentration in duplicate aliquots (50 μL) of the soluble fraction was determined by the Folin-Ciocalteau method (Andersen & Todd, 1968) and expressed as 4-coumaric acid equivalents as previously described (Booker & Miller, 1998).

Duplicate residue samples were analyzed with a CHN elemental analyzer (Model 2400, Perkin-Elmer Inc., Analytical Services Laboratory, Department of Soil Science, North Carolina State University, Raleigh, NC, USA) to determine C and N concentrations. Duplicate 1 g residue samples were used for determination of acid-detergent fiber (ADF), cellulose, and acid-insoluble ash-free lignin concentrations (Soil and Forage Analysis Laboratory, University of Wisconsin, Marshfield, WI, USA) (Van Soest, 1963).

Laboratory microcosm assay

The glass jar method was used for determinations of potential C and N mineralization (Torbert *et al*., 1998). Dried, 20 g sieved (2 mm mesh) soil samples obtained from a fallow section of the field where the experiment was conducted were mixed with duplicate 100 mg samples of residue components obtained from each year of the experiment and placed in plastic containers. Deionized water was added to adjust soil water content (equivalent to −20 kPa at a bulk density of 1.2 Mg m^{-3}). Containers were placed in sealed glass jars with 20 mL of water (humidity control) and a 20 mL vial of 1 N NaOH (CO_2 trap). The jars were incubated in the dark at 25 °C for up to 60 days. The incubation period was based on previous studies with soybean residues that showed that mineralization of readily available C and N was completed by 60 days and that further release was relatively low (Henning *et al*., 1996; Torbert *et al*., 1998, 2000). Plant residue remaining after 60 days would consist primarily of humic material that would decompose slowly. Carbon dioxide in NaOH traps was determined after incubation for 30 and 60 days by titrating excess base with 1 N HCl in the presence of $BaCl_2$. Potential C mineralization is the difference between CO_2-C captured in sample traps and blanks. Soil inorganic N was extracted with 2 M KCl and measured by standard colorimetric procedures (Torbert *et al*., 1998). Potential N mineralization is the difference between inorganic N content of samples and blanks (soil only).

Litter bag decomposition assay

Four samples of leaves (30 g), petioles (30 g), stems (50 g), and pod husks (50 g) from each chamber were placed in separate 25 cm × 25 cm fiberglass 1.4 mm × 1.7 mm mesh litter bags. Litter bags segregated by original treatment blocks were placed in shallow excavations in an adjacent fallow field and covered with 3–5 cm of sandy loam soil (pH 5.5, 0.2% N, 1.2% C) on 8 December 1999 and 13 December 2000. After 20 weeks, the litter bags were recovered. The 20-week incubation period allowed us to examine the extent of residue decomposition after an over-wintering period

Table 2 Environmental conditions during incubation of litter bags in the 2-year experiment (1999–2001)

Year	Month	T_{max} (°C)	T_{min} (°C)	T_{avg} (°C)	T_{soil} (°C)	Rain (cm)
1999	December	14	2	8	9	6
2000	January	10	−1	5	6	13
	February	15	2	8	6	17
	March	19	8	14	11	8
	April	22	9	14	14	8
	May (1–5)	28	12	27	16	0
2000	December	9	−1	3	4	3
2001	January	12	0	6	4	3
	February	19	7	13	8	8
	March	16	4	10	9	16
	April	24	10	17	16	6

Monthly average maximum (T_{max}), minimum (T_{min}), and average (T_{avg}) air temperatures and rainfall were measured on site. Soil temperature (T_{soil}) was obtained from a site 10 km north-west of our location.

to determine how much plant matter would be left at the start of the next season. Litter bag contents were dried to a constant weight at 60 °C, sieved using a 2 mm mesh screen to remove soil debris, and weighed. Duplicate 2 g samples of litter bag residues were ashed (500 °C for 12 h) and weighed to obtain estimates of ash-free dry mass (AFDM). Contamination of residue AFDM by organic matter in the soil was considered minimal because average AFDM of the soil in which the litter bags were buried was 2.4%. Monthly temperatures and rainfalls during the litter bag incubation periods are shown in Table 2.

Statistical analysis

The treatments consisted of factorial combinations of two CO_2 levels and two O_3 levels, and the treatments were assigned to chambers in a randomized complete block design. There were three replicate chambers for each treatment combination in each year of the experiment. Assay results from chamber samples were averaged for use as a chamber replicate value. For each block, a separate randomization of treatments to chambers was done in each year of the experiment. Results from the 2 years were combined for the statistical analysis ($n = 6$ for each treatment in the 2-year experiment). Data were checked for homogeneity of variance. Treatment effects and means for residue biomass, residue chemistry, and litter bag assays were analyzed using a three-factor mixed model for the effects of year, CO_2, and O_3 with random block, block by year, and within plot terms (SAS Proc Mixed) (Littell *et al*., 1996).

To investigate relationships between residue decomposition and its initial chemistry, regression analysis of the remaining AFDM in the litter bags was performed on the separate residue chemistry parameters (SAS Institute Inc., 2001; Hoorens *et al*., 2003). Regressions were performed on data normalized by calculating the numerical difference between the average value of a measured component and each value of that component in each year of the experiment. Normalized data from each year of the experiment were combined for the regression analyses.

Treatment effects and means for the laboratory microcosm assays were estimated using a repeated measures model in which chambers constituted the whole plots and incubation period was the repeated factor (SAS Proc Mixed) (Littell *et al*., 1996). The model included main effects and interactions of CO_2 and O_3 at the whole plot level with random block, block by year, and chamber effects in addition to within plot error. The sub-plot part of the model included the incubation effect.

Results

Harvest biomass

Postharvest residues were composed of 20% leaf, 15% petiole, 33% stem, and 32% pod husk by dry mass among treatments. (Leaf and petiole residue data came from the experiment conducted in 2000. In 1999, quantitative estimates of senesced leaves and petioles could not be obtained because of high winds and rainfall associated with a hurricane whereas stems and pods were generally intact.) Average dry mass of the residue components was 28–56% higher in the elevated CO_2 treatment and 15–46% lower in the elevated O_3 treatment compared with the control treatment (Fig. 1, Table 3). In the elevated CO_2 and O_3 treatment, average dry mass of residue components was 21–24% higher than the control. Thus, the decrease in residue mass in the elevated O_3 treatment relative to the control treatment was attenuated by elevated CO_2.

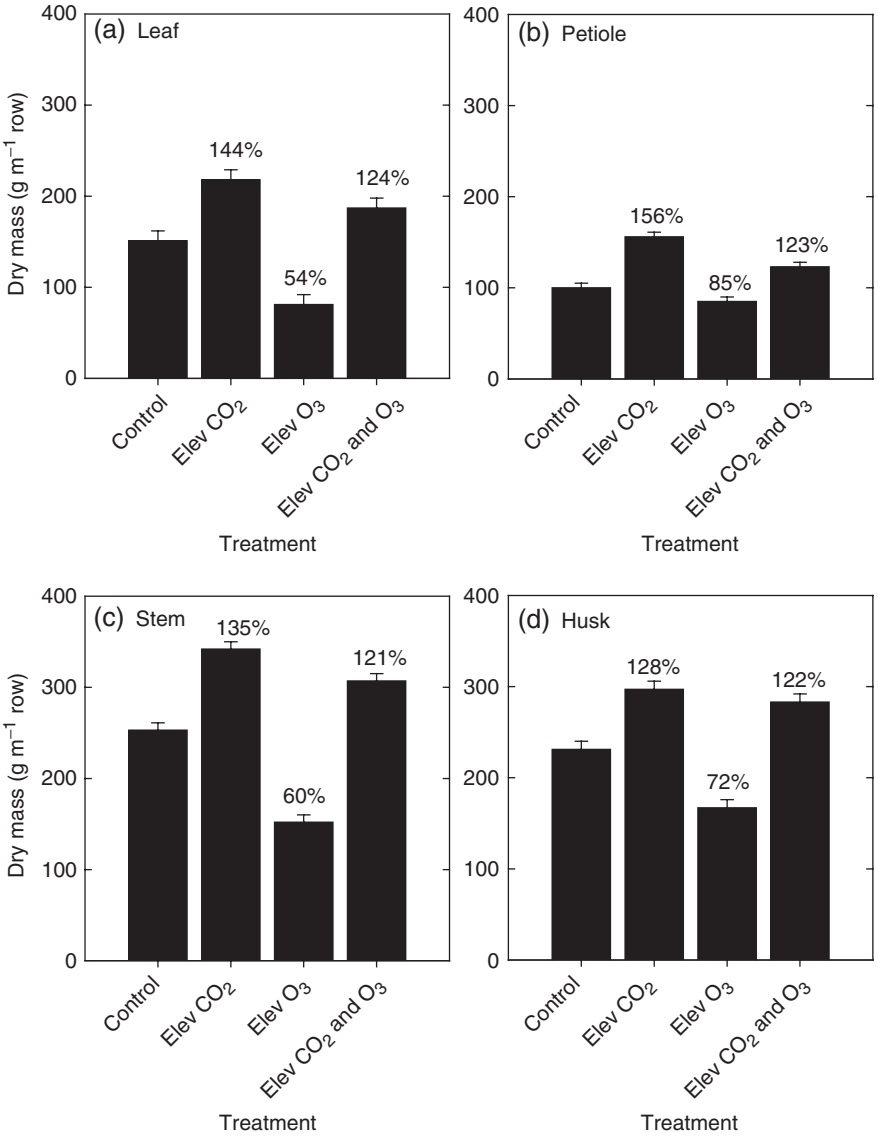

Fig. 1 Effects of elevated CO_2 and O_3 on leaf (a), petiole (b), stem (c), and pod husk (d) harvest residue biomass per square meter of row. The treatments were: (a) charcoal-filtered air and ambient CO_2 (Control); (b) charcoal-filtered air plus 344 μmol CO_2 mol^{-1} (Elev CO_2); (c) nonfiltered air plus 1.5 times ambient O_3 and ambient CO_2 (elevated O_3); and (d) nonfiltered air plus 1.5 times ambient O_3 and 344 μmol CO_2 mol^{-1} (Elev CO_2 and O_3). Values are means ± SE of six replicate chambers per treatment (three replicates per year except for leaves and petioles, for which there were only three replicates from 1 year of the experiment (2000)). Values above the bars indicate percent of the control treatment.

Amelioration of O_3 effects by elevated CO_2 was less effective for leaf and petiole residues compared with stem and husk residues, as indicated by the lack of statistically significant CO_2 by O_3 interactions for leaf and petiole residues (Table 3).

Plant residue chemistry

Among the plant organ residues analyzed, the most noteworthy treatment effects occurred on leaf residue chemistry (Table 4). Starch and soluble sugar concentrations in leaf residues from the elevated O_3 treatments were 25–54% lower than in residue from the control treatment. Soluble phenolic concentrations were 15% higher in leaf residues from the elevated CO_2 treatments. Nitrogen concentrations were 7–15% higher in leaf residues from the elevated CO_2, elevated O_3, and combined gas treatments. Ash-free lignin concentrations were 24–35% higher in leaf residues from these treatments as well.

Petiole, stem, and pod husk residues from plants treated with elevated CO_2 contained slightly higher

Table 3 Probabilities of CO_2 and O_3 treatment effects on leaf, petiole, stem, and pod husk postharvest dry masses

Effect	df	Leaf	Petiole	Stem	Husk
Year	1	ND	ND	*	*
CO_2	1	***	***	***	***
O_3	1	**	**	***	***
$CO_2 \times O_3$	1	NS	NS	**	*

Leaf and petiole probabilities were based on data from one growing season (2000). Significant treatment effects and interactions are indicated as $^*P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. ND, not determined; NS, not significant.

ADF and cellulose concentrations compared with the control (Table 4). Otherwise, elevated CO_2 treatment effects on residue chemistry were generally not statistically significant. Elevated O_3 treatment effects on petiole, stem, and husk residues were minor with the exception of husk residue N concentration, which was increased by 16% (Table 4).

Treatment effects on biomass production, however, influenced the content of chemical constituents on an areal basis (Table 5). Calculation of aboveground plant chemical composition based on the dry mass and composition of each residue organ assayed indicated that elevated CO_2 increased the mass per square meter of total nonstructural carbohydrates (sugar plus starch concentrations), soluble phenolics, N, cellulose, and lignin by 24–46%. Added O_3 decreased the mass per square meter of these constituents by 30–48%, while elevated CO_2 largely ameliorated the added O_3 effect, with the exception of total nonstructural carbohydrate concentrations. Nonstructural carbohydrate concentrations were lower in the elevated CO_2 and O_3 treatment compared with the control.

Laboratory microcosm assays

Mineralized C and N increased between the 30- and 60-day incubation periods for all residue samples (Figs 2 and 3, Table 6). However, treatment effects on C mineralization were not statistically significant (Fig. 2, Table 6).

Petiole and stem residues from all elevated gas treatments immobilized up to 30% more N after incubation for 30 days compared with the control (Fig. 3, Table 6). After incubation for 60 days, there was a net release of N from soils containing petiole and stem residues from the control treatment whereas N remained immobilized in the elevated gas treatments. There was no significant treatment effect of elevated CO_2 or O_3 on N mineralization of leaf and husk residues.

Litter bag decomposition assay

Respective monthly air and local soil temperatures were similar during litter bag incubation periods in 1999–2000 and 2000–2001, although the 1999–2000 winter months were wetter than in 2000–2001 (Table 2). However, 20–57% less leaf, petiole, and stem AFDM was recovered after incubation in the soil for 20 weeks in the second year of the experiment compared with the first, while twice as much pod husk AFDM was found.

Percent remaining AFDM was 48% higher for leaf residues from the elevated O_3 and combined gas treatments compared with the control treatment after burial in the soil for 20 weeks (significant O_3 and $CO_2 + O_3$ effects) (Fig. 4, Table 7). Percent remaining petiole and stem AFDM was also slightly higher in the elevated gas treatments. There were no statistically significant treatment effects on husk residue decomposition.

Remaining leaf AFDM was significantly correlated with initial residue starch, sugar, N, ADF, and ash-free lignin concentrations, although the adjusted coefficients of determination for sugar, N and ADF were relatively weak (Table 8). Initial leaf residue starch and sugar concentrations were negatively related to remaining leaf residue AFDM, meaning that residues with higher initial starch and sugar concentrations decomposed to a greater extent. In contrast, leaf residue N, ADF, and ash-free lignin concentrations were positively related to remaining residue AFDM, indicating that leaf residues with higher concentrations of these components decomposed to a lesser extent. Starch and ash-free lignin concentrations were positively correlated with remaining stem AFDM, although the coefficients of determination were low (Table 8). Regressions of petiole and husk residue AFDM on measured residue components were not statistically significant.

Discussion

Results of this study support the growing consensus that the decomposition of crop plant residues is not greatly affected by growth in elevated concentrations of CO_2 (Torbert *et al*., 1995, 2000; Henning *et al*., 1996; Booker *et al*., 2000; Norby *et al*., 2001; Prior *et al*., 2004). Recent decomposition experiments with residues from woody species exposed to elevated CO_2 support this finding as well (Norby *et al*., 2001; Finzi & Schlesinger, 2002; Kainulainen *et al*., 2003). Elevated CO_2 usually had small effects on residue chemistry, except for lower N and higher lignin concentrations, although neither

Table 4 Effects of elevated CO_2 and O_3 on starch, soluble sugar, phenolic, N, C : N ratios, acid detergent fiber (ADF), cellulose, and ash-free lignin concentrations of leaf, petiole, stem, and pod husk residues at harvest

Treatment	Starch (mg g^{-1})	Sugar (mg g^{-1})	Phenolics (mg g^{-1})	N (mg g^{-1})	C/N	ADF (%)	Cellulose (%)	Lignin (%)
Leaf								
Control	6.5 ± 0.6	4.2 ± 0.4	11.5 ± 0.7	12.1 ± 0.4	40 ± 1	31.0 ± 1.2	14.3 ± 0.5	6.6 ± 0.3
Elevated CO_2	6.6 ± 0.6	3.4 ± 0.4	13.0 ± 0.7	13.0 ± 0.4	42 ± 1	31.6 ± 1.2	14.6 ± 0.5	8.2 ± 0.3
Elevated O_3	3.0 ± 0.6	2.1 ± 0.4	11.3 ± 0.7	13.9 ± 0.4	37 ± 1	35.8 ± 1.2	13.6 ± 0.5	8.9 ± 0.3
Elevated CO_2 and O_3	4.9 ± 0.6	2.9 ± 0.4	13.1 ± 0.7	13.1 ± 0.4	41 ± 1	32.0 ± 1.2	14.6 ± 0.5	8.4 ± 0.3
Year	NS	**	**	**	***	**	**	*
CO_2	†	NS	*	NS	*	NS	NS	NS
O_3	***	**	NS	**	NS	*	NS	**
$CO_2 \times O_3$	NS	*	NS	*	NS	†	NS	**
Petiole								
Control	1.0 ± 0.2	1.7 ± 0.3	4.0 ± 0.2	10.4 ± 0.5	43 ± 2	56.2 ± 0.9	43.2 ± 0.9	10.3 ± 0.5
Elevated CO_2	0.4 ± 0.2	1.9 ± 0.3	3.6 ± 0.2	9.8 ± 0.5	45 ± 2	58.2 ± 0.9	44.4 ± 0.9	10.8 ± 0.5
Elevated O_3	0.8 ± 0.2	1.1 ± 0.3	3.0 ± 0.2	10.6 ± 0.5	44 ± 2	58.6 ± 0.9	45.5 ± 0.9	10.2 ± 0.5
Elevated CO_2 and O_3	0.8 ± 0.2	1.1 ± 0.3	3.1 ± 0.2	10.2 ± 0.5	43 ± 2	60.6 ± 0.9	46.6 ± 0.9	11.1 ± 0.5
Year	NS	*	*	**	**	*	*	NS
CO_2	NS	NS	NS	NS	NS	*	NS	†
O_3	NS	*	***	NS	NS	*	*	NS
$CO_2 \times O_3$	NS	NS	†	NS	NS	NS	NS	NS
Stem								
Control	0.3 ± 0.1	1.5 ± 0.2	4.6 ± 0.3	8.9 ± 0.5	50 ± 3	64.4 ± 0.8	48.3 ± 0.6	14.2 ± 0.3
Elevated CO_2	0.2 ± 0.1	1.0 ± 0.2	4.5 ± 0.3	7.5 ± 0.5	57 ± 3	65.0 ± 0.8	49.0 ± 0.6	14.2 ± 0.3
Elevated O_3	0.6 ± 0.1	1.2 ± 0.2	3.9 ± 0.3	7.6 ± 0.5	57 ± 3	65.4 ± 0.8	48.6 ± 0.6	14.9 ± 0.3
Elevated CO_2 and O_3	0.5 ± 0.1	1.2 ± 0.2	3.8 ± 0.3	7.9 ± 0.5	56 ± 3	67.4 ± 0.8	50.1 ± 0.6	15.4 ± 0.3
Year	NS	†	*	NS	NS	†	*	NS
CO_2	NS	NS	NS	NS	NS	*	*	NS
O_3	†	NS	*	NS	NS	**	NS	**
$CO_2 \times O_3$	NS	NS	NS	†	NS	NS	NS	NS
Husk								
Control	0.7 ± 0.2	1.2 ± 0.2	3.9 ± 0.1	13.1 ± 0.8	32 ± 2	41.8 ± 0.4	32.8 ± 0.4	7.1 ± 0.2
Elevated CO_2	0.4 ± 0.2	1.4 ± 0.2	3.7 ± 0.1	14.6 ± 0.8	30 ± 2	42.8 ± 0.4	33.6 ± 0.4	7.4 ± 0.2
Elevated O_3	0.2 ± 0.2	1.4 ± 0.2	4.4 ± 0.1	15.2 ± 0.8	28 ± 2	41.3 ± 0.4	31.9 ± 0.4	7.5 ± 0.2
Elevated CO_2 and O_3	0.2 ± 0.2	1.7 ± 0.2	4.6 ± 0.1	15.6 ± 0.8	26 ± 2	42.2 ± 0.4	32.8 ± 0.4	7.6 ± 0.2
Year	NS	*	**	*	*	**	**	NS
CO_2	NS	NS	NS	NS	NS	*	†	NS
O_3	NS	NS	***	†	*	NS	†	*
$CO_2 \times O_3$	NS	NS	†	NS	NS	NS	NS	NS

Values are means ± SE of six replicate chambers for each treatment combination. Significant treatment effects and interactions are indicated as $^{\dagger}P \leq 0.1$; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.
NS, not significant.

factor consistently affected C mineralization or mass loss rates in past studies (Booker *et al.*, 2000; Norby *et al.*, 2001). Therefore, the effects of elevated CO_2 on decomposition might not depend so much on changes in residue chemistry as they do on increased residue input. Increased biomass production is a common effect of elevated CO_2 on various crop species (Kimball, 1983; Rogers & Dahlman, 1993; Fiscus *et al.*, 2001; Ainsworth *et al.*, 2002; Prior *et al.*, 2004). Aboveground residue production was increased by elevated CO_2 in both years of our experiment, although leaf residue decomposition also was decreased, possibly because of higher lignin concentration. The higher leaf residue N concentrations apparently had little influence on decomposition rates.

Although the elevated CO_2 and O_3 treatments had relatively minor effects on residue chemistry, treatment effects on soybean growth resulted in potentially important differences in inputs of residue chemical components to the soil on an areal basis (Table 5). For example, aboveground residues of soybean plants treated with elevated CO_2 contained approximately 3,

Table 5 Effects of elevated CO_2 and O_3 on total plant residue biomass, total nonstructural carbohydrates (TNC), soluble phenolics, N, cellulose, and ash-free lignin concentrations on an areal basis (data were from the 2000 experiment only; leaf and petiole residues were not obtained in 1999 because of damage from inclement weather)

Treatment	Residue biomass ($g\,m^{-2}$)	TNC ($g\,m^{-2}$)	Phenolics ($g\,m^{-2}$)	N ($g\,m^{-2}$)	Cellulose ($g\,m^{-2}$)	Lignin ($g\,m^{-2}$)
Control	757 ± 36	3.7 ± 0.4	5.8 ± 0.3	8.2 ± 0.7	260.3 ± 1.1	75.6 ± 3.0
Elevated CO_2	1027 ± 36 (136%)	4.6 ± 0.4 (124%)	8.5 ± 0.3 (146%)	11.1 ± 0.7 (135%)	360.4 ± 1.1 (138%)	105.3 ± 3.0 (139%)
Elevated O_3	455 ± 36 (60%)	1.4 ± 0.4 (38%)	3.0 ± 0.3 (52%)	5.7 ± 0.7 (70%)	153.4 ± 1.1 (59%)	48.4 ± 3.0 (64%)
Elevated $CO_2 \times O_3$	893 ± 36 (118%)	3.2 ± 0.4 (86%)	7.2 ± 0.3 (124%)	10.0 ± 0.7 (122%)	312.3 ± 1.1 (120%)	98.6 ± 3.0 (131%)
CO_2	***	**	***	*	***	***
O_3	***	**	***	**	***	***
$CO_2 \times O_3$	NS	NS	NS	NS	*	*

Values are means ± SE of three replicate chambers for each treatment combination. Significant treatment effects and interactions are indicated as $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$.
Values in parentheses indicate percent of the control treatment. NS, not significant.

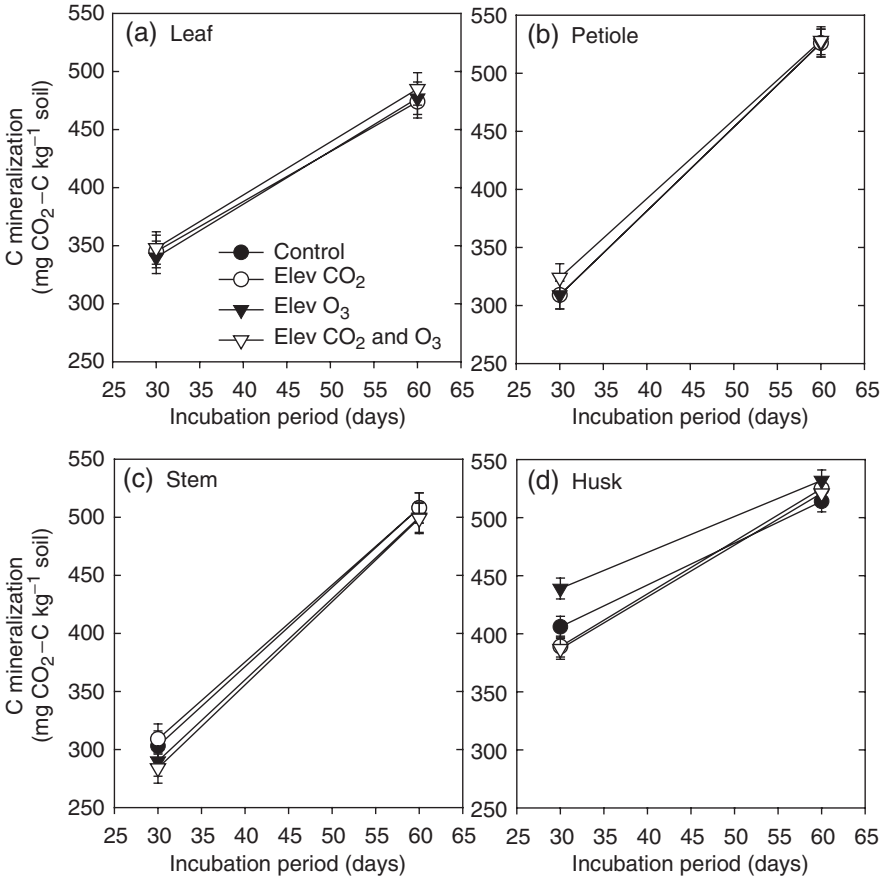

Fig. 2 CO_2-C mineralization rate of leaf (a), petiole (b), stem (c), and pod husk (d) residues in a laboratory microcosm experiment. Residues were obtained from soybean plants treated with reciprocal combinations of CO_2 and O_3 concentrations as described in the legend for Fig. 1. Values are means ± SE of six replicate chambers per treatment.

100, and 25 more grams per square meter of N, cellulose, and lignin, respectively, than residues from the control treatment. In contrast, residues from the elevated O_3 treatment contained about 2.5, 107, and 27 fewer grams per square meter of these constituents, respectively, compared with residues from the control.

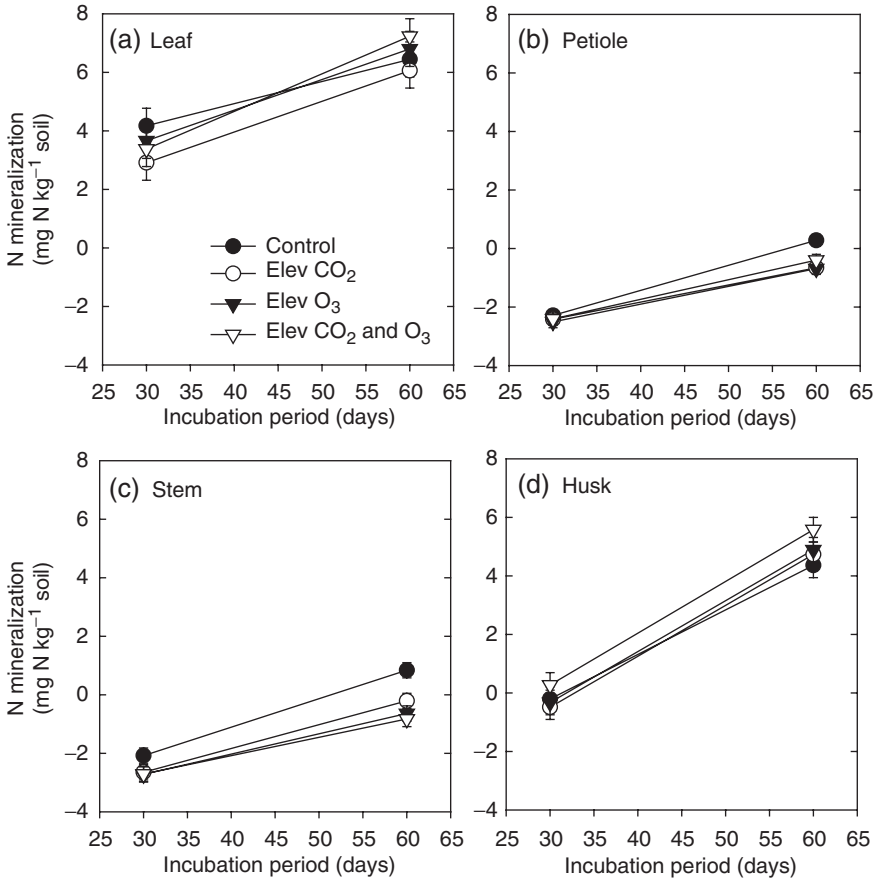

Fig. 3 N mineralization rate of leaf (a), petiole (b), stem (c), and pod husk (d) residues in a laboratory microcosm experiment. Residues were obtained from soybean plants treated with reciprocal combinations of CO_2 and O_3 concentrations as described in the legend for Fig. 1. Values are means ± SE of six replicate chambers per treatment.

Table 6 Probabilities of CO_2 and O_3 treatment effects on C and N mineralization rates of leaf, petiole, stem, and pod husk residues following incubation in a laboratory microcosm experiment for 30 and 60 days (IncubPeriod)

Effect	df	Leaf C	Petiole C	Stem C	Husk C	Leaf N	Petiole N	Stem N	Husk N
Year	1	*	**	*	**	**	**	†	NS
CO_2	1	NS	NS	NS	*	NS	NS	†	NS
O_3	1	NS	NS	NS	NS	NS	*	**	NS
$CO_2 \times O_3$	1	NS	NS	NS	†	NS	**	NS	NS
IncubPeriod	1	***	***	***	***	***	***	***	***

Significant treatment effects and interactions are indicated as $^{\dagger}P \leq 0.1$; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.
NS, not significant.

Torbert *et al*. (2004) found that elevated CO_2 increased whole-plant residue N content by 19–37% in soybean. Soybean is a legume capable of symbiotic N_2 fixation, and previous studies (Zanetti *et al*., 1996; Kimball *et al*., 2002) found that elevated CO_2 increased nodulation and N_2 fixation by legumes. Increased N_2 fixation accounted for the majority of the increased N level in legume plants treated with elevated CO_2 (Zanetti *et al*., 1996; Torbert *et al*., 2004). This would suggest that the increased levels of N in the total aboveground residue per square meter from the elevated CO_2 treatments in our study were obtained in part by increased N_2 fixation. One result of increased residue production and higher levels of recalcitrant material such as lignin being added to the soil is that soil C sequestration should increase, a response anticipated to occur with

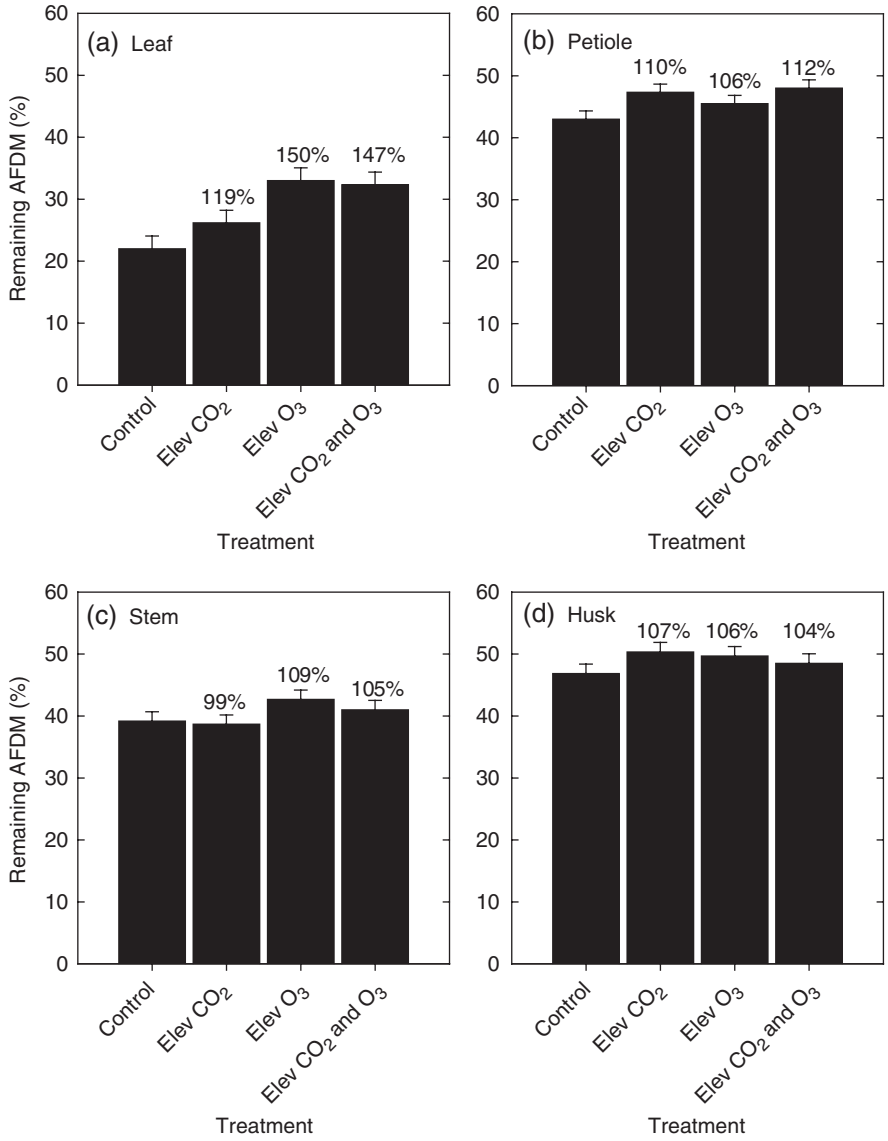

Fig. 4 Percent remaining leaf (a), petiole (b), stem (c), and pod husk (d) ash-free dry mass (AFDM) in litter bags after incubation in the soil for 20 weeks. Residues were obtained from soybean plants treated with reciprocal combinations of CO_2 and O_3 concentrations as described in the legend for Fig. 1. Values are means ± SE of six replicate chambers per treatment. Values above the bars indicate percent of the control treatment.

Table 7 Probabilities of CO_2 and O_3 treatment effects on percent remaining ash-free dry mass (AFDM) of leaf, petiole, stem and pod husk residues following incubation in buried litter bags for 20 weeks

Effect	df	Leaf	Petiole	Stem	Husk
Year	1	†	*	**	**
CO_2	1	NS	**	NS	NS
O_3	1	***	NS	*	NS
$CO_2 \times O_3$	1	†	NS	NS	NS

Significant treatment effects and interactions are indicated as $^{\dagger}P \leq 0.1$; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.
NS, not significant.

increasing concentrations of atmospheric CO_2. The opposite effect is likely to result from exposure of plants to increasing concentrations of tropospheric O_3, although a decline in residue biomass input could be offset by increases in phenolic polymer concentrations.

In our study, elevated O_3 suppressed aboveground residue production and thus litter input to the soil. Although O_3-treated residues did not affect C mineralization rate in the microcosm assay, leaf residue decomposition was significantly less in the 20-week-long litter bag assay compared with the control. The decreased decomposition was associated with lower nonstructural carbohydrate and higher ash-free lignin

concentrations of the leaf residues. Even though added O_3 decreased leaf residue mass loss, decomposition of other aboveground residues was generally unaffected. Leaf residues only comprised about 20% of the total residue biomass assayed so treatment effects on mass loss of total aboveground residues were relatively small.

As a matter of clarification, the commonly used methods for lignin quantification (Klason lignin and ADF lignin (Van Soest, 1963)) do not actually measure lignin quantity, but rather lignin and lignin-like materials. The assays quantify lignin along with other nonhydrolysable products (Jin *et al*., 2003). Alkaline nitrobenzene oxidation, ozonation, and methoxyl assays indicated that lignin concentration in decomposing leaf litters from several tree species was much less than that indicated by Klason residues and tended to remain unchanged during a 1-year decomposition experiment (Jin *et al*., 2003). The Klason residue is representative of undegradable material, not necessarily lignin. Similar results were found for O_3-treated soybean in which increased phenolic polymer levels were found that were not attributable to increased lignin concentrations (Booker & Miller, 1998). Therefore, the material classified as lignin in our study likely overestimated the actual lignin concentration and represented lignin plus other nonhydrolysable components.

Both elevated CO_2 and elevated O_3 increased N immobilization in soils containing stem and petiole residues. Similar effects on N mobility were found in previous studies with soybean, sorghum, and cotton residues treated with elevated CO_2 (Torbert *et al*., 1995; Henning *et al*., 1996; Torbert *et al*., 2000). However, it is unlikely that increased N immobilization will impact long-term crop productivity. Retardation of N cycling in agricultural soils would not be expected to affect crop production because N inputs are usually adequate under current agronomic practices. Nitrogen mobility is usually linked to corresponding soil C dynamics, which may affect soil quality and N levels in agroecosystems over the long term (Torbert *et al*., 2000). Although agricultural soils are generally N-enriched, enhanced N immobilization suggests that N limitation may be possible at certain stages of crop growth, which highlights the importance of effective N management (Torbert *et al*., 2000).

Elevated CO_2 nearly negated the inhibitory effect of elevated O_3 on residue biomass production, but otherwise chemistry, mineralization, and mass loss rates of residues from the combined gas treatment were similar to residues from the elevated CO_2 and elevated O_3 treatments. The $CO_2 \times O_3$ interaction, however, is gas concentration, species, and cultivar dependent. Lower or higher concentration of either gas has the potential to alter the magnitude of the interaction. For example, enhanced biomass production of soybean, cotton, rice (*Oryza sativa* L.), and wheat by twice-ambient CO_2 concentration was much less in charcoal-filtered air ($25\,nmol\,O_3\,mol^{-1}$) compared with nonfiltered air ($50\,nmol\,O_3\,mol^{-1}$) (12 h daily averages) (Fiscus *et al*., 2001). In our study, total residues were 38% higher in the elevated CO_2 treatment compared with the control treatment, whereas they were 85% higher in the combined gas treatment compared with the elevated O_3 treatment. Plant sensitivity to the treatment gases is an integral factor in this interaction as well. For example, some snap bean (*Phaseolus vulgaris* L.) and potato (*Solanum tuberosum* L.) cultivars are so sensitive to O_3 that twice-ambient CO_2 concentration did not fully protect them from injury by 1.5 times ambient O_3 (Heagle *et al*., 2002, 2003).

From the standpoint of experimental design, we recognize that use of charcoal-filtered and nonfiltered air in combination with ambient and elevated levels of CO_2 might have the theoretical potential to confound the experiment. While it is conceivable, we have no evidence to suggest there is some unknown component in nonfiltered air, as opposed to charcoal filtered air, that would alter plant responses to added O_3 or CO_2. Past research indicates that O_3, SO_2, and NO_2 are responsible for up to 90% of the crop loss caused by air pollution (Heck *et al*., 1982). However, ambient NO_2 and SO_2 concentrations are below phytotoxic levels at

Table 8 Adjusted coefficients of determination of the normalized remaining leaf and stem ash-free dry mass (AFDM) in the litter bags on their respective normalized residue chemistry

Residue	Starch	Sugar	Phenolic	N	C/N	ADF	Cellulose	Ash-free lignin
Leaf	−0.36***	−0.20*	NS	+ 0.17*	NS	+ 0.15*	NS	+ 0.30**
Stem	+ 0.21*	NS	NS	NS	NS	NS	NS	+ 0.11†

All regressions of normalized petiole and husk residue AFDM on their normalized chemistries were not statistically significant. Positive or negative relationships are indicated by plus (+) or minus (−) signs. Significant regressions are indicated as $^{\dagger}P \leq 0.1$; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. AFDM, ADF, acid detergent fiber; NS, not significant.

our location. There is also entrainment of ambient air in all chambers so plants are exposed to some ambient air even in chambers receiving charcoal-filtered air. Thus, there is no reason to presume that use of charcoal-filtered and nonfiltered air would lead to unrecognized interactions in this experiment.

In conclusion, the major impact of elevated CO_2 on decomposition processes and soil C sequestration will likely occur through increased residue input. Soybean residue input, on an areal basis, will contain more C, N, and phenolic compounds. However, ambient O_3 has the potential to affect these responses through complex interactions involving lowered residue production, slower leaf residue decomposition, and differences in O_3 sensitivity among species and cultivars.

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